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Radial basis function neural networks for modeling growth rates of the basidiomycetes *Physisporinus vitreus* and *Neolentinus lepideus*

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Abstract A radial basis function (RBF) neural network was developed and compared against a quadratic response surface (RS) model for predicting the specific growth rates of the biotechnologically important basidiomycetous fungi, *Physisporinus vitreus* and *Neolentinus lepideus*, under three environmental conditions: temperature (10–30 °C), water activity (0.950–9.998), and pH (4–6). Both the RBF network and polynomial RS model were mathematically evaluated against experimental data using graphical plots and several statistical indices. The evaluation showed that both models gave reasonably good predictions, but the performance of the RBF neural network was superior to that of the classical statistical method for all three data sets used (training, testing, full). Sensitivity analysis revealed that of the three experimental factors the most influential on the growth rate of *P. vitreus* was water activity, followed by temperature and pH to a lesser extent. In contrast, temperature in particular and then water activity were the key determinants of the development of *N. lepideus*. RBF neural networks could be a powerful technique for modeling fungal growth behavior under certain parameters and an alternative to time-consuming, traditional microbiological techniques.

Keywords Radial basis function neural network · *Physisporinus vitreus* · *Neolentinus lepideus* · Response surface model · Growth rate

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Introduction

Recent investigations have shown that, in combination with conventional histological and analytical methods, wood-decay fungi have many valuable biotechnological applications in the pure and applied wood sciences (Messner et al. 2002; Mai et al. 2004; Schwarze 2008). Alterations in the cell wall structure and/or the distribution of the cell wall constituents are reflected in the plasticity of the wood degradation modes of these fungi (Deflorio et al. 2005; Schwarze 2008). The specificity of their enzymes and the mild conditions under which degradation proceeds make them potentially suitable agents for wood modification, e.g., biopulping and bioremediation (Majcherczyk and Hüttermann 1988; Messner et al. 2002).

The biotechnological process of bioincising is a promising approach to improve the uptake of preservatives and wood-modification substances by refractory wood as a result of selective degradation of bordered pits by the white-rot fungus *Physisporinus vitreus* (Pers.: Fr.) P. Karst. (Schwarze and Landmesser 2000; Schwarze et al. 2006). Furthermore, because of its exceptional pattern of degradation, *P. vitreus* is successfully used to improve the acoustic properties of the tonewood of Norway spruce wood (*Picea abies* L.) used for musical instruments by selectively delignifying the secondary walls without affecting the

middle lamellae, even at advanced stages of degradation (Schwarze et al. 2008; Spycher et al. 2008).

The growth rate (GR) of *P. vitreus* in relation to temperature, water activity (a_w), and pH was compared in the present work with that of the brown-rot fungus *Neolentinus lepideus* ((Fr.: Fr.) Redhead and Ginns, common name = *Lentinus lepideus*), another important basidiomycete that is used for bioremediation and detoxification of preservative-treated waste wood because of its creosote tolerance and ability to degrade, among other compounds, toxic poly aromatic hydrocarbon compounds (Shuen and Buswell 1992; Cerniglia 1997; Samson et al. 1998; Richter et al. 2003).

Improving the effectiveness of wood modification by minimizing variable performance and optimizing development of the fungi demands an understanding of how abiotic parameters affect their lag phase and GR. The classical method of testing these parameters involves varying the level of each parameter over a certain range, while maintaining the other test variables constant. This strategy is time consuming, requires a large number of experiments, and does not include interactive effects among parameters on the process. In order to overcome these problems, predictions of fungal growth can be obtained using mathematical modeling approaches (Skinner et al. 1994).

Response surface (RS) methodology has been widely used in predictive microbiology for quantitative assessment of the effects of various environmental factors on various ascomycetes (Sparringa et al. 2002; Panagou et al. 2003; Lahlali et al. 2005, 2006; Begoude et al. 2007; Schubert et al. 2009a). The modeling approach has also been used to predict the growth of several bacteria (García-Gimeno et al. 2005; Carrasco et al. 2006; Dong et al. 2007) and to optimize the growth conditions of the alga *Nannochloropsis oculata* (Spolaore et al. 2006).

As an alternative to the traditional methods of statistical regression, artificial neural networks (ANNs) have recently been developed and found widespread acceptance as data-driven modeling techniques because of their remarkable ability to model highly complex and nonlinear problems in many fields of engineering and science (Basheer and Hajmeer 2000; Hajmeer et al. 2006). ANNs normally have no restriction on the type of relationship between the growth parameters (input patterns) and the desired output, whereas regression-based models require that the order of the model is stated beforehand (Panagou and Kodogiannis 2009). In contrast, ANNs directly explore the knowledge contained in the input–output patterns by adjusting the parameters of the nonlinear ANN topology as the input–output patterns are repeatedly presented to the network (Hajmeer et al. 2006). When the system is supervised trained using an appropriate training data set, it can then be used to predict GR for different growth conditions within the initial experimental range (Panagou and Kodogiannis 2009). Radial basis function (RBF) neural networks, a variant of ANNs, were recently used by Panagou et al. (2007) to successfully predict the GR of the ascomycete, *Monascus ruber* van Tieghem, for the first time.

To the best of our knowledge, RBF neural network methodology has not been used for *P. vitreus* and *N. lepideus*, so the aim of the present work was (1) to develop a RBF neural network to predict GR under different experimental conditions, (2) to compare the predictive accuracy of the RBF approach with RS methodology, and (3) to determine the relative importance of the three parameters (temperature, a_w, and pH) on the development of *P. vitreus* and *N. lepideus*.

Materials and methods

Microorganisms

The cultures of *P. vitreus* (642) and *N. lepideus* (340) are maintained and deposited at the EMPA, Materials Science and Technology, Switzerland. The cultures were identified microscopically and additionally the internal transcribed spacer ITS1–5.8S–ITS2 region of the rDNA was amplified and sequenced for *P. vitreus* (EMBL, accession no. FM202494) and *N. lepideus* (EMBL, accession no. FM995531). For the experiments, the initial inoculum was taken from cultures that had been growing on malt extract agar medium (MEA, Oxoid, Darmstadt, Germany) in Petri dishes at 4 °C for no more than 6 months.

Medium

The a_w of the basic MEA medium (0.998) was adjusted by adding calculated amounts of nonionic solute (glycerol) to obtain nine different moisture levels (0.998, 0.990, 0.982, 0.974, 0.970, 0.966, 0.958, 0.955, and 0.950) (Dallyn and Fox 1980), measured with an osometer (OM 801, Vogel, Giessen, Germany). The medium was buffered with 0.1 mmol l^{-1} NaH_2PO_4 and the final pH of the medium was adjusted to 4 or 5 with 1 mol l^{-1} HCL, or to 6 with 1 mol l^{-1} NaOH, prior to autoclaving.

Radial growth rate assessment

Using a cork borer, mycelial discs (9 mm) were removed under sterile conditions from 10-day-old colony cultures of *P. vitreus* and *N. lepideus* growing on 2% MEA at 22 °C and then inoculated at the center of Petri dishes containing the test media. After inoculation, the Petri dishes were sealed in polyethylene bags to prevent water loss and incubated for 20 days at 10, 15, 20, 25, and 30 °C. A total of 135 full factorial experiments (five temperatures × nine

a_w × three pH) were performed. Three replicates were used for each combination of experimental conditions and each experiment was conducted twice (n=810). The radial growth of each mycelial colony was measured daily in two predetermined directions, without opening the Petri dishes, until the plates were completely colonized. The radial GR (mm day^{-1}) for each a_w, temperature, and pH combination was obtained from linear regression slopes of the temporal growth curves. The lag phase (time required for macroscopically measurable growth) was also assessed for each experimental combination. Combinations of environmental conditions in which no growth occurred were not taken into account for model fitting, resulting in a new database of 75 datasets for *P. vitreus* and 117 datasets for *N. lepideus*. The new database of *P. vitreus* was divided into a training set with a total of 61 data (81%) and a testing (validation) set with 14 data (19%); the same procedure for *N. lepideus* resulted in a training set with a total of 95 data (81%) and a testing (validation) set with 22 data (19%). The testing set was used as the basis for determining the predictive accuracy and was selected randomly, whereas great care was taken to ensure that the training set adequately represented the experimental data of both fungi (Panagou et al. 2007).

Response surface methodology

The GRs (mm day^{-1}) were investigated by analysis of variance. Statistical significance was judged at the level of P<0.05. Whenever analysis revealed significant difference, Duncan's multiple range test for separation of means was performed. The mean estimates of GR were then fitted to a quadratic RS model to describe the single and combined effects of temperature, pH, and a_w (Schubert et al. 2009b):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad (1)$$

where Y = response (GR), β_0 coefficient = off-set term called intercept, x_i = independent variables related to the factors, β_i = linear coefficients, β_{ij} = second-order interaction coefficients, β_{ii} = quadratic coefficients, and ε = error of the model.

Interactions between factors could appear either as an antagonistic effect (negative coefficient) or a synergistic effect (positive coefficient). Interpretation of the data was based on a positive or negative effect on the response and statistical significance of the coefficients (P<0.05). The higher the absolute value of a linear coefficient (β_x), the greater the influence of the corresponding factor.

Radial basis function neural network

The following brief introduction to RBF neural networks is based on the detailed description and explanation by Panagou et al. (2007). As illustrated in Fig. 1, the RBFs are embedded in a two-layer feed-forward neural network, with a single hidden layer in which the nodes are Gaussian kernels and a linear output layer. The RBF is represented by the following equation:

$$f(x) = \sum_{i=1}^{nr} w_i \, \phi(\|x - c_i\|) \qquad (2)$$

where x = input vector, w_i = weight of the output layer, $\phi(\cdot)$ = Gaussian kernel, $\|\cdot\|$ = the Euclidean norm, c_i = center of the ith kernel, and n_r = total number of kernels. The Gaussian kernel $\varphi(\cdot)$ is defined as:

$$\phi(\|x - c_i\|) = e^{\left(-\frac{\|x-ci\|^2}{2\sigma^2}\right)} \qquad (3)$$

where σ = width of the kernel. In Eq. (3), let $\phi = \exp\left(-0.8326\|x - c_i\|/\mathrm{SPREAD}^2\right)$. If $\mathrm{SPREAD} = \|x - c_i\|$, then ϕ=0.5. Spread determines the width of the area input space to which each neuron in the first layer responds (Demuth et al. 2008). The larger the spread, the smoother the function approximation will be. A too large spread means a lot of neurons will be required to fit a fast changing function. A too small spread means many neurons will be required to fit a smooth function and the network may not generalize well. The key to successful implementation of the network is to find suitable centers for the Gaussian functions, so the appropriate selection of the training data set (supervised) and training algorithm are crucial for the success of the RBF model.

In the following simulations and experiments, training of the RBF neural network was performed by the NEWRB function in Matlab® Software (ver. 7.7.0 MathWorks, R2008a). The final RBF network contained 50 neurons for *P. vitreus* and 75 neurons for *N. lepideus* and one spread parameter for all input variables (σ=1) for both fungi. The values of the output weights in the linear layer of the network for both fungi are presented in Table S1 (supplementary material).

Criteria for comparison

For an appropriate comparison of the robustness and reliability of the RBF neural network and the RS model, both were developed and validated with the same training, testing, and full data sets. To assess the fitting and predictive accuracy of the models, the datasets were mathematically evaluated by calculating the following evaluation criteria: coefficient of determination (R^2) (Box and Draper 1987), root mean squared error (RMSE), standard error of prediction (SEP) (García-Gimeno et al. 2005; Zurera-Cosano et al. 2006), mean relative percentage error (MRPE), mean absolute percentage error (MAPE) (Jeyamkondan et al. 2001), bias factor (B_f), accuracy factor

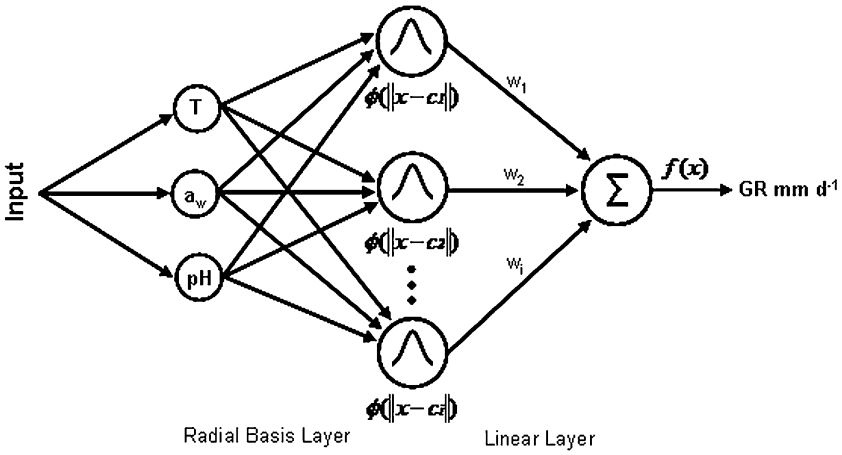

Fig. 1 Architecture of the radial basis function neural network

(A_f) (Ross 1996), and the proportion of the relative error (pRE) (Oscar 2005). In addition, a graphical comparison was performed to illustrate the accuracy of the proposed models.

Sensitivity analysis

The determination of the relative significance of model input parameters, ranking the variables in order of importance, was performed by sensitivity analysis on the trained RBF neural network. The sensitivity of an output parameter, $Out_{j=1,2,\ldots j}$, to an input parameter, $In_{i=1,2,\ldots ni}$, was defined as the normalized ratio between variations caused in Out_j by variations introduced in In_i (Noble et al. 2000) and is represented by the following equation:

$$NS = \left(dOut_j/dIn_i\right)\left(In_i/Out_j\right) \quad (4)$$

Results

The combined abiotic factors of a_w × temperature × pH tested in this study significantly influenced the mycelial development and growth behavior of the wood-decay fungi *P. vitreus* and *N. lepideus* in different ways. As illustrated in Fig. 2, changing a_w at different steady-state temperatures and pH levels affected performance by decreasing the GR of both fungi, but particularly of *P. vitreus*. The a_w range in which growth of *P. vitreus* occurred was 0.998–0.970, whereas within the 20-day incubation period no growth was detected at $a_w \leq 0.966$. For *N. lepideus*, however, growth occurred at a_w 0.998–0.958 at all temperatures and pH levels tested within the incubation period. At $a_w = 0.955$, *N. lepideus* showed detectable growth at temperatures ≥15 °C, regardless of pH. The GR of both fungi was higher at $a_w = 0.998$ and at all temperatures, slightly higher at pH 5 than at 4 or 6, diminished with decreasing a_w and temperatures and at suboptimal conditions the effect was practically absent. Both fungi had their growth optima at pH 5 and at the highest a_w (0.998), but at different temperatures. The most supportive temperature for growth was 25 °C for *P. vitreus* and 30 °C for *N. lepideus*, provided that the a_w was high enough (≥0.970 for *P. vitreus* and ≥0.955 for *N. lepideus*). Growth of both fungi decreased with decreasing temperature, but *P. vitreus* showed detectable growth at all temperatures at $a_w \geq 0.982$, whereas the growth of *N. lepideus* decreased rapidly with decreasing temperatures even at optimal a_w levels (Fig. 2).

Results of the mathematical evaluation of the predictive accuracy of the RBF neural network and statistical RS model are presented in Table 1 and Figs. 3, 4, and 5 for the three data sets (training, testing, full) for *P. vitreus* and *N. lepideus*. As shown in Table 1, both models revealed a good predictive accuracy for *P. vitreus* and *N. lepideus*, but the RBF neural network was able to predict the GR of both fungi, and for all data sets, with greater accuracy than the polynomial model (RS), as inferred by the indices. With the training data sets in particular, the RBF neural network predictions were far superior to those of the RS model for both fungi, as judged by the relevant indices, especially SEP and pRE (Table 1). Figures 3, 4, and 5 show the bias (observed vs. predicted GR mm day^{-1}), residuals plots, and RE for both models, for all data sets and for *P. vitreus* and *N. lepideus*, respectively. The bias from the RBF neural network was generally closer to the line of equity ($y = x$) compared with the RS model for all data sets and for both fungi (Fig. 3). The residual plots revealed that the spread of the residuals of the RBF neural network for both fungi was narrower to zero than for the RS model and additionally, the residuals were distributed symmetrically around zero, indicating no systematic tendency to either the positive or negative side of the graph. The analysis of the RE for all data sets and for both fungi revealed that the RBF neural network was widely within the proposed range from −0.3 fail-safe to 0.15 fail-dangerous under all experimental conditions (optimal–suboptimal), whereas the RS model

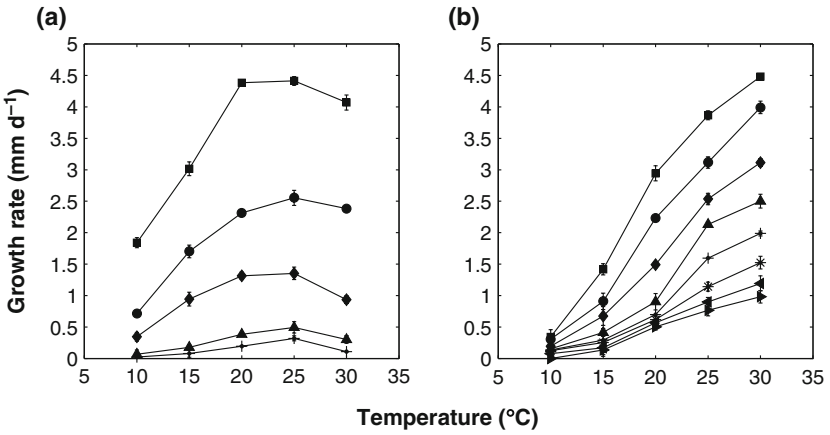

Fig. 2 Effect of water activity and temperature at pH 5 on the radial growth rate of *Physisporinus vitreus* (**a**) and *Neolentinus lepideus* (**b**). Water activity levels are 0.998 (■), 0.990 (●), 0.982 (♦), 0.974 (▲), 0.970 (+), 0.966 (*), 0.958 (◄), and 0.955 (►). *Error bars* show standard error (SE) of the estimated parameters. Where the *bars* are not shown, they are smaller than the symbol size

showed good accuracy at supportive growth conditions, but at suboptimal conditions, close to the growth/no growth interface, the values were almost out of the proposed range for all data sets and both fungi (Fig. 5).

The results of multiple regression analysis, which provided estimates of the model coefficients for the training and full data sets of *P. vitreus* and *N. lepideus*, are listed in Table 2. The descending order of the model coefficients of *P. vitreus* was a_w (β_2) > temperature (β_1) > pH (β_3), showing that the influence of a_w on growth was greater than that of temperature and pH for the training and full data sets. In contrast, the regression analysis for *N. lepideus* revealed the highest model coefficient of temperature (β_1), followed by a_w (β_2) and pH (β_3) for the training and full data sets. The obtained results of the regression analysis were confirmed by sensitivity analysis of the relative significance of model input parameters. Table 3 shows that a_w was the most influential (sensitive) parameter affecting the GR of *P. vitreus* in all data sets (training, testing, full), followed by temperature and pH to a lesser extent, whereas the growth of *N. lepideus* in all data sets was particularly influenced by temperature, followed by a_w and pH.

Discussion

Highly efficient modification or detoxification of wood by *P. vitreus* or *N. lepideus* is based on optimal development of the mycelium, including the lag phase, hyphal elongation, and branching. Mycelial growth is greatly influenced by

Table 1 Comparison of the evaluation indices of a radial basis function neural network (RBF) and a statistical model (RSM)

Statistical index	Model	Data set of *P. vitreus*			Data set of *N. lepideus*		
		Training	Testing	Full	Training	Testing	Full
Coefficient of determination (R^2)	RBF	1.0000	0.9959	0.9985	0.9991	0.9947	0.9982
	RSM	0.9910	0.9932	0.9902	0.9899	0.9882	0.9896
Root mean squared error (RMSE)	RBF	0.0095	0.0914	0.0738	0.0519	0.1203	0.0717
	RSM	0.1831	0.1384	0.1901	0.1710	0.1692	0.1705
Standard error of prediction (SEP%)	RBF	0.6182	9.9923	5.4081	3.8912	10.799	5.5619
	RSM	13.293	15.131	13.936	12.809	15.197	13.216
Mean relative percentage error (MRPE)	RBF	−0.3149	2.3616	0.4996	−0.1935	−2.2686	−0.5549
	RSM	−8.3817	−1.9266	−9.7973	1.2234	9.6239	1.8825
Mean absolute percentage error (MAPE)	RBF	2.8212	12.308	2.8521	3.8258	8.8269	4.8496
	RSM	47.781	34.705	46.132	26.228	34.798	25.576
Bias factor (B_f)	RBF	0.9988	0.9434	0.9876	1.0005	1.0159	1.0030
	RSM	1.0479	1.1211	1.0959	1.0384	0.7656	0.9604
Accuracy factor (A_f)	RBF	1.0298	1.1616	1.0348	1.0389	1.0923	1.0496
	RSM	1.3894	1.2362	1.3298	1.2113	1.6479	1.2870
Portion of relative error (pRE)	RBF	0.9672	0.8125	0.9600	1.0000	0.9167	0.9829
	RSM	0.5902	0.6250	0.5867	0.6947	0.6250	0.7009

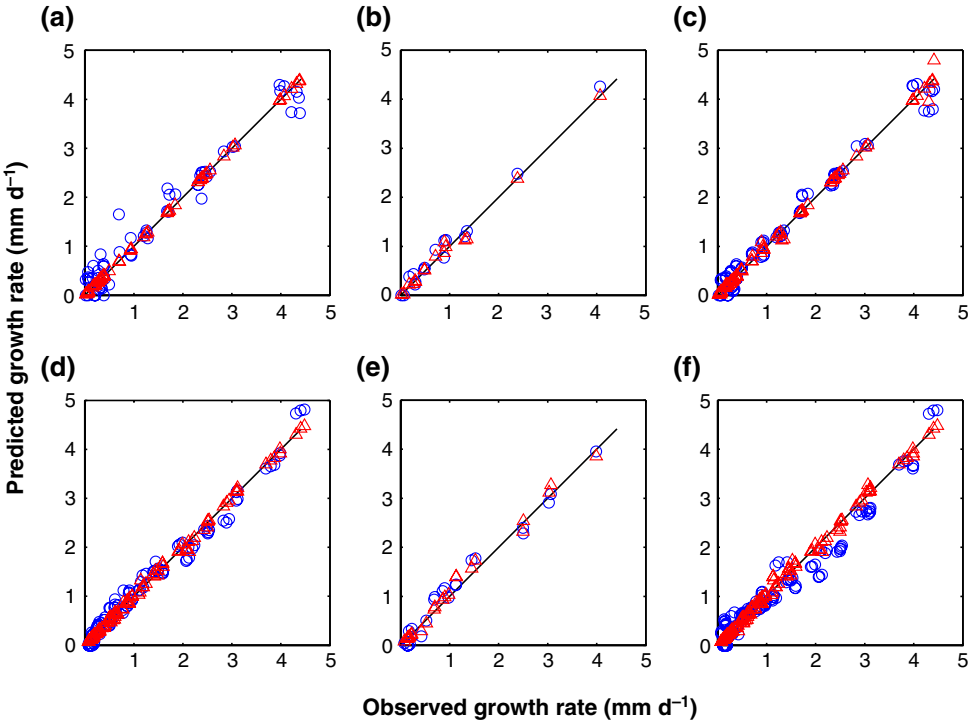

Fig. 3 Comparison of predicted and observed values of growth rate for *Physisporinus vitreus* (**a**–**c**) and *Neolentinus lepideus* (**d**–**f**) according to the radial basis function neural network (Δ) and the response surface model (○) for the training, testing, and full data sets

environmental conditions (Rayner and Boddy 1988), so for optimal use of these fungi in biotechnological processes it is necessary to broaden our knowledge of the influence of physical, physico-chemical, or chemical factors. In this study, two mathematical methods of prediction were compared to identify the effect of temperature, a_w, and pH on the specific GRs of *P. vitreus* and *N. lepideus*. One approach was a classical method of statistical analysis, using RS methodology, and the other was based on ANN technology, using a RBF neural network.

Comparison of the two models was based on both graphical plots and evaluation indices (Table 1, Figs. 3, 4, and 5). The predictions of the generated quadratic polynomial model and of the RBF neural network correlated properly with the experimental observations, although the RBF neural network had better agreement for the training, testing, and full data sets for both fungi. The overall predictive capability of a model is commonly expressed by the coefficient of determination (R^2), which measures how much of the variability of the observed response value is attributable to experimental factors and their interactions (Box and Draper 1987). In the present work, the calculated values of the coefficient were markedly high for all data sets of both fungi (RS model >98%; RBF >99%). However, the R^2 value alone is not a measure of a model's accuracy, because by adding a variable to the model the R^2 will increase, regardless of whether the additional variable is significant or not (Baş and Boyaci 2007). Thus, it is possible for models that have large values of R^2 to yield poor predictions of new observations or estimates of the mean response (Myers and Montgomery 1995). For this reason, further evaluation of the model's performance (verification) was quantified by calculating the RMSE, SEP% (García-Gimeno et al. 2005; Zurera-Cosano et al. 2006), A_f, B_f (Ross 1996), and pRE (Oscar 2005). RMSE provides a measure of the goodness-of-fit of a model to the data used to produce it (Box and Draper 1987). Table 1 shows the low values for RMSE for all data sets of *P. vitreus* and *N. lepideus*, which indicated a good fit of the experimental data by both models, although the RBF neural network showed in all cases lower values than the RS model. The index also provides information about how consistent the model would be in the long term (Lou and Nakai 2001).

The SEP index is the relative deviation of the mean prediction values and has the advantage of being independent of the magnitude of the measurements (García-Gimeno et al. 2005). Based on this index, the RBF neural network was far superior to the polynomial model for all data sets of both fungi (Table 1).

The MRPE and MAPE indices are similar to the bias (B_f) and accuracy factors (A_f) proposed by Ross (1996). Both indices, but MAPE in particular, indicated a better performance for the RBF neural network.

The prediction bias (B_f) was calculated as described by Ross (1996), but the boundaries of the acceptable prediction zone were equivalent to those proposed by Ross et al. (2000) for unacceptable values of B_f <0.70 or >1.15. Because the B_f value does not provide an indication of the average accuracy of the estimates, the A_f was also calculated. Although a value

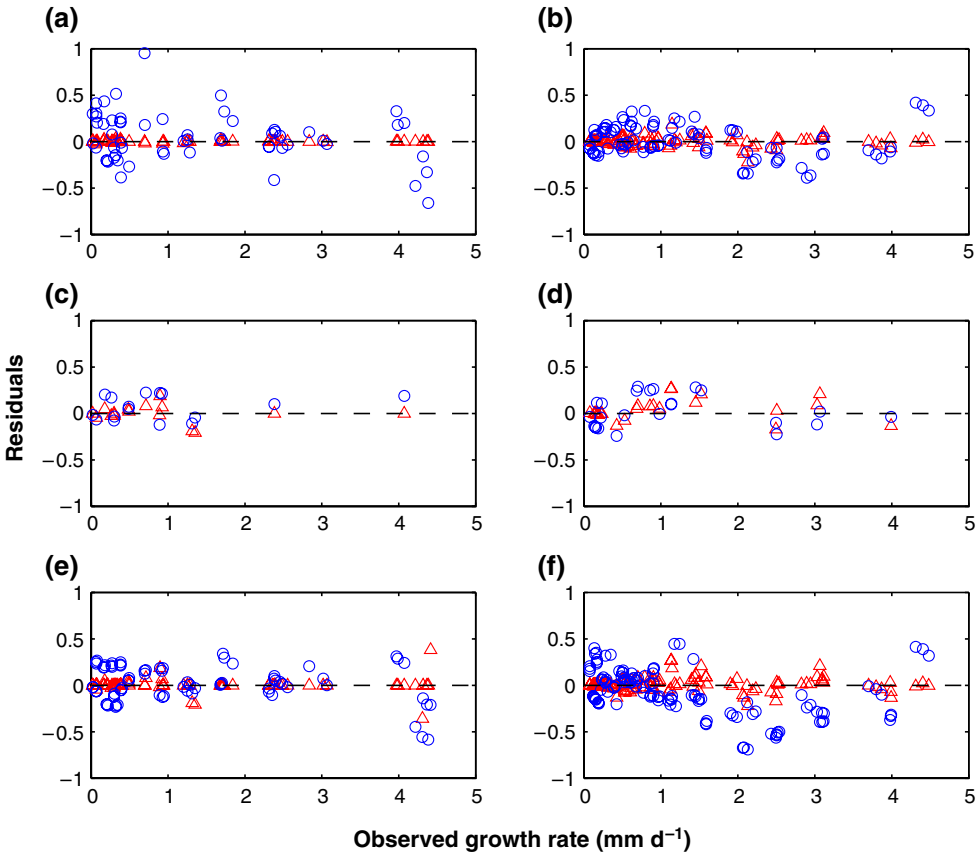

Fig. 4 Comparison of residuals (predicted–observed growth rate) of *Physisporinus vitreus* (**a**, **c**, **e**) and *Neolentinus lepideus* (**b**, **d**, **f**) according to the radial basis function neural network (Δ) and the response surface model (○) for the training, testing, and full data sets

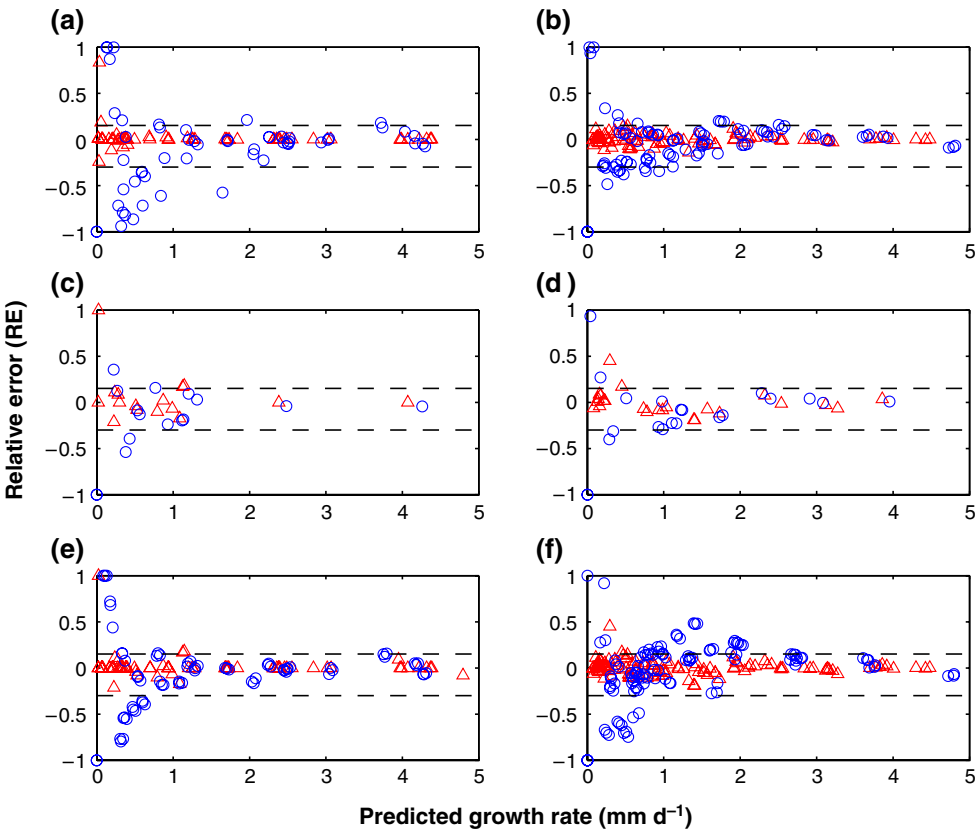

Fig. 5 Relative error (RE) plots with an acceptable prediction zone from RE −0.3 (fail-safe) to 0.15 (fail-dangerous) for comparison of observed and predicted values of specific growth rates of *Physisporinus vitreus* (**a**, **c**, **e**) and *Neolentinus lepideus* (**b**, **d**, **f**) according to the radial basis function neural network (Δ) and the response surface model (○) for the training, testing, and full data sets

Table 2 Model coefficients of the quadratic model and their significant effects on the radial growth rate (mm day^{-1}) of *Physisporinus vitreus* and *Neolentinus lepideus*

Coefficients		Data set of *P. vitreus*		Data set of *N. lepideus*	
		Training	Full	Training	Full
Intercept	β_0	1.4212**	1.4461**	1.2846**	1.2427**
T	β_1	0.5571**	0.5708**	1.3165**	1.2935**
a_w	β_2	1.6250**	1.6444**	1.0523**	1.0671**
pH	β_3	0.0321**	$0.0059^{n.s}$	$0.0079^{n.s}$	0.0125**
T^2	β_4	−0.5358**	−0.6019**	0.0518**	0.0959**
a_w^2	β_5	0.6848**	0.7062**	0.2417**	0.2598**
pH^2	β_6	−0.0270*	−0.0353*	−0.0541**	−0.0422**
$T \times a_w$	β_7	0.5080**	0.5467**	0.8674**	0.8713**
$T \times$ pH	β_8	$-0.0140^{n.s.}$	$-0.0053^{n.s.}$	$0.0104^{n.s.}$	$-0.0004^{n.s.}$
$a_w \times$ pH	β_9	0.0559**	$0.0038^{n.s.}$	$0.0125^{n.s.}$	0.0153*

a_w water activity, T temperature
**Highly significant ($P<0.0001$), *significant ($P<0.05$), $^{n.s.}$ not significant ($P\geq0.05$)

of 1 indicates that there is perfect consistency between all predicted and observed values, Ross et al. (2000) considered an A_f value to be acceptable with an increase of up to 0.15 (15%) for each variable included in the model. In the present case, the calculated B_f and A_f values for the RBF neural network and RS model, and for all data sets of both fungi, were in the defined acceptable range, which indicates high agreement, except for the RS model with the testing data set of *N. lepideus*, which revealed an unacceptable A_f value of 1.6479 (Table 1).

However, these indices (A_f, B_f) do not generate absolute measures of performance, because both are specific to the data sets used in the evaluation, so care should be taken with their interpretation (García-Gimeno et al. 2005). According to Dalgaard and Jørgensen (1998), a limitation of A_f and B_f, which are ratios of the observed and predicted values, is that they cannot be calculated for cases in which no growth is predicted by the model and growth is observed or vice versa. In the present study, at suboptimal growth conditions, the RS model predicted no growth, but detectable growth occurred (data not shown). Excluding these cases leads to an overestimation of the model's performance, so it is recommended that for both indices (A_f, B_f) there should be additional calculation of the pRE (Oscar 2005). For cases in which observed or predicted values are infinity or zero, the RE assigns a value of −1 for graphical presentation, which is an important feature of the acceptable prediction zone method because it allows the inclusion of no-growth cases in the calculation of the performance factor (Oscar 2005). In the present work, the boundaries of the acceptable prediction zone of the pRE were equivalent to those proposed by Ross et al. (2000), which are 0.7 (fail-safe) to 1.15 (fail-dangerous). Models with pRE >0.700 are considered to provide prediction with acceptable bias and accuracy (Oscar 2005). The RBF neural network revealed for all data sets of both fungi acceptable values of >0.700, whereas the RS model revealed mostly unacceptable values of <0.700 (Table 1). The aforementioned remarks could explain this discrepancy of the indices and confirm the better performance of the RBF neural network compared with the statistical approach for modeling growth under in vitro conditions.

Predictive microbiology studies the behavior of microorganisms under different conditions and can help identify critical growth parameters, the most influential parameters, and thus the optimal cultivation/application conditions. In the present work, the selection of experimental conditions was based on the data of Schubert et al. (2009b), but included testing of a wider range of temperatures (10–30 °C) and more a_w values (nine) to observe the response of the

Table 3 Results of the sensitivity analysis ranking the parameters in order of importance for the growth rate (mm day^{-1}) of *Physisporinus vitreus* and *Neolentinus lepideus*

Fungus	Data set	Parameters		
		Temperature (°C)	Water activity (a_w)	pH
P. vitreus	Training	2	1	3
	Testing	2	1	3
	Full	2	1	3
N. lepideus	Training	1	2	3
	Testing	1	2	3
	Full	1	2	3

fungi even under extreme environmental conditions. The sensitivity analysis of the RBF neural network and the multiple regression analysis confirmed the experimental results and showed that a_w is a particularly key determinant of the development of *P. vitreus* (Schmidt et al. 1996, 1997; Schubert et al. 2009b), whereas for *N. lepideus* temperature was the most influential parameter. The pH factor was the least important for both fungi, as the chosen experimental range of pH was found mainly in the optimum region for fungal growth. Both fungi showed their growth optimum under the highest a_w value (0.998) and at pH 5, although *N. lepideus* revealed a relative tolerance to water stress. Griffin (1977) and Anagnost (2007) categorized most wood-decay fungi as hydrophilic organisms, which require $a_w \geq 0.97$, but *N. lepideus* showed detectable growth at $a_w = 0.955$, provided the temperature was >10 °C. The most supportive temperature for *P. vitreus* was 20–25 °C and at higher temperatures (>25 °C), the GR declined. However, at all a_w levels, *N. lepideus* showed an increased GR with increasing temperature (10–30 °C), but no final conclusion about its optimal growth temperature can be stated and further studies should include temperatures >30 °C.

The differences in the ecological demands of both fungi are expressed by their appearance on timber used in service. The strong dependence of *P. vitreus* on high moisture levels, its preferential colonization and degradation of water-saturated wood (wood moisture content WMC >100%), even though fungal growth is normally impeded due to the lack of oxygen at WMC >90%, highlights the remarkable biological capacity of this fungus to degrade timber in the special habitat of cooling towers (Schmidt et al. 1996, 1997). *N. lepideus* showed an ability to sustain water stress in wood which coincides with its affinity to colonize creosoted railway sleepers, telegraph poles, and indoor timber, where ambient temperature is high and the relative humidity low (Cartwright and Findlay 1958; Rayner and Boddy 1988).

The results of this preliminary study demonstrate that modeling approaches, particularly the RBF neural network, combined with sensitivity analysis, represent a significant advance in defining the optimal environmental conditions for fast initial mycelial growth, which is important for the future optimization and success of biotechnological applications using *P. vitreus* and *N. lepideus*.

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